Molecular characterization of Begomoviruses and its resistance through RNAi silencing in *Nicotiana benthamiana*

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Introduction

Geminiviruses are characterized by the twinned, small (ca. 18-30 nm), quasi-icosahedral morphology of the virion particles and by their genomes consisting of one or two molecules of ssDNA 2.5- 3.0 kb in length. During the last decade, the geminiviruses were classified into the *Geminiviridae* family (Rybicki, 1994), which contains four genera: *Mastrevirus*, *Curtovirus*, *Topocuvirus*, and *Begomovirus*, classified according to their host range, genome organization, and vector species (Palmer & Rybicki, 1998; Fauquet *et al.*, 2000).

Begomoviruses cause serious diseases in many crop plants, which is undoubtedly a primary reason for the strong research interest they have generated. Till date, begomoviruses have been reported from at least 16 different groups of crop in India (Dasgupta *et al.*, 2012). The interaction of begomoviruses with the vector whiteflies (*Bemisia tabaci)*, a crucial step in the spread of begomoviruses in the field, also needs to be carefully looked at. White-fly commonly found in tropical and sub-tropical regions, and in greenhouses of temperate areas. The Northern India supports such type of climates for the breeding of whitefly. *B. tabaci* was first described in the genus Aleyrodes in 1889 and was first reported as a pest in 1919 in India (Husain and Trehan, 1993). So far, ~2000 virus species have been approved, divided into 6 orders, 87 families and 349 genera (Brown *et al.*, 2012), reflecting their economic importance and enormous diversity resulting from their widespread geographic distribution and host adaptation.

The transmission of viruses by whiteflies that has the widest impact on global food production, including on key crops such as tomatoes, beans, cassava, cotton, cucumbers, potatoes, sweet potatoes, melons, gourds, and squashes etc. An interesting aspect of Indian begomoviruses is their overlapping host range (for example, tomato-infecting begomoviruses have been reported from plants as diverse as chili, cotton and mentha and cassava-infecting begomoviruses have been reported from bitter gourd and jatropha). One of the major factors responsible for this overlapping host range could be the polyphagous nature of the vector whitefly and the mixed cropping systems prevalent in the country.

The first ever DNA satellite discovered, referred to as ToLCV-sat, was isolated from tomato plants infected with the monopartite begomovirus ToLCV (Dry *et al.*, 1997). This circular satellite is small (682 nucleotides) with no open reading frames and little sequence similarity to its helper virus, with the exception of sequences within the apex of two stem-loop structures, one containing the conserved geminivirus TAATATTAC motif and the other containing a putative ToLCV Rep binding motif (Behjatnia *et al.*, 1998). ToLCV-sat is not required for ToLCV infectivity and had no effect on the symptoms induced by the helper virus but it is dependent on the helper begomovirus for its replication and encapsidation and thus it represents a true satellite.

All full length betasatellites encode a single complementary sense gene which is positionally and functionally conserved and referred to as *βc1*. The typical length of the product predicted to be encoded by *βc1* is 118 amino acids with a predicted molecular weight of

13.7kDa. The precise function of betasatellites and its βC1 protein in pathogenesis of viruses although it has been proposed that DNA β may play a direct or an indirect role in replication, facilitating movement, or countering host defense response

Begomovirus infected plants often exhibit yellowing of leaf veins, yellow mosaics patterns, curling of fractional or complete leaves, leaf distortion, and decrease in leaf size and stunting in plants. Numerous crops are now genetically engineered with genes obtained from geminiviruses, with the hope of finding significant stable pathogen derived resistant to these viruses.

PCR primers based on conserved sequences can be used to clone and sequence the N-terminal sequences of the CP and other genes of the geminiviruses; this sequence is sufficient to classify a virus isolate. A possible taxonomic structure for geminiviruses has been proposed after considering the sequence comparisons and biological properties (Malla *et al.*, 1995).

The sequence analysis using CP genes and betasatellites of the proposed begomovirus complexes suggested that inter-specific recombination has resulted in remarkable diversity among their geographical isolates and could be a major cause of the emergence of new geminivirus diseases.

In an investigation to study the contribution of the βC1 protein of Begomovirus in effective gene silencing of βC1 fused to the GUS in *Nicotiana benthamiana* infiltrated with *Agrobacterium tumefaciens* that harbors the intron-hairpin RNA construct aimed at the βC1.

Along with this also study about the RNAi mediated immunity in *N* benthamiana can be engineered by Agrobacterium-mediated transformation harboring binary vector construct carrying βC1 gene in sense and anti-sense orientation with short intron, to produce siRNAs in plant targeting the mRNA of Begomovirus βC1. This strategy of expressing a gene encoding intron-spliced RNA can induce PTGS with almost 100% efficiency when directed against viruses (Smith *et al.*, 2000).

Aims of the study

This thesis focuses on the study of the diversity amongst the begomoviruses, naturally infecting crops in India and their resistance through RNAi silencing mechanism.

The specific aims of this study were:

- Identification of the *begomoviruses* and its possible alternative hosts in India, and molecular diversity of monopartite begomovirus coat protein and betasatellite associated with different crop species in India.
- Identification and genomic characterization of a begomovirus and satellites infecting *Spinacia oleracea in* northern India.
- Molecular and biological characterization of helper begomoviruses and associated satellites in *Cymopsis tetragonoloba* from Rajasthan India.
- Evaluation of transient expression and shut down of gene expression of βC1 of begomovirus in transgenic and non-transgenic *N.benthamiana.*
- Involvement of the individual gene product (βC1) of begomovirus in symptoms induction and RNA silencing.

Review of literature:

Viruses are obligate intracellular parasites and they are dependent on the molecular machinery of their host for their replication. Viruses belonging to the family *Geminiviridae* are continue to have worldwide impact on agricultural production to this date. Begomoviruses can be broadly divided into two groups based on phylogenetic analysis, the Old World viruses (those originating from Europe, Africa, Asia and Australasia) and the New World viruses (those originating from the Americas; Padidam *et al.,* 1999; Paximadis *et al.,* 1999; Rybicki, 1994). All New World begomoviruses are bipartite consisting of two separately encapsidated genomic components, known as DNA-A and DNA-B (Brown *et al.*, 2012), whereas some monopartite begomoviruses have been introduced from the Old World (OW) (Zhang & Ling, 2011) and the majority of these associate with recently identified satellite molecules; very few are bipartite. Recently alphasatellites have been found associated with bipartite begomoviruses from the NW (Paprotka *et al.*, 2010; Romay *et al.*, 2010).

The current classification by International Committee on Taxonomy of Viruses (ICTV) classifies viruses into families (-viridae), genera (-virus) and species on the basis of: the nature of their genome, RNA or DNA; single or double stranded genome and positive/negative sense nucleic acid. So far, ~2000 virus species have been approved, divided into 6 orders, 87 families and 349 genera (Brown *et al.*, 2012). It has been suggested that there is a minimum of 10-100 viruses for each host species of microorganism, plant and animal. More than 1,000 different viruses can infect humans (Norrby, 2008). In plants, viruses are casual agents of ~47% of all emerging diseases (Anderson *et al.*, 2004). Currently, there are 20 families with plant-infecting viruses sub grouped into 90 genera, which comprise ~800 plant virus species (Brown *et al.*, 2012). Generally, plant viruses have isometric or helical symmetry and vary in size ranging between 17 and 2000 nm (Hull, 2002). Over 90% of plant viruses have ssRNA genomes while less than 10% of plant viruses have DNA, including both single-stranded (ss) and double-stranded (ds) DNA. However caulimoviruses (family *Caulimoviridae*) are dsDNA viruses whereas nanoviruses (*Nanoviridae*) and geminiviruses (*Geminiviridae*) are ssDNA viruses.

Begomoviruses are the largest group of geminiviruses (Briddon *et al.,* 2001; Mansoor *et al.,* 2008), includes monopartite (single genomic component of approx. 2.8kb), bipartite (two genomic component of approx. equal size known as DNA A and DNA-B; Stanley *et al.,* 2005). The DNA-A component of bipartite begomoviruses and the genomes of monopartite begomoviruses encode four ORFs, termed *(a)c1* to *(a)c4* (*c1* to *c4* in monopartite viruses), on the complementary-sense strand. (A)C1 to (A)C3 encode the Rep, the transcriptional activator protein (TrAP), and the replication enhancer protein (REn), respectively. Replication-associated protein (Rep) of about 41kDa, is encoded by ORF C1 (also called AC1 or AL1) in all geminiviruses and, due to its similarities with rolling circle DNA replication initiator proteins of some prokaryotic plasmids (Koonin and Ilyina, 1992), has been called "Replication associated protein" (Laufs *et al.,* 1995b).

Begomoviruses encoded two genes in complementary sense strand, the V1 (Coat protein) and V2. However, the begomoviruses from the New World lack the AV2. Mutation analysis has shown that the (A)V2 protein is involved in the movement of begomoviruses (Padidam *et al.,* 1996). Coat protein (CP) is a multifunctional protein required for a range of functions associated with encapsidation, accumulation of viral ssDNA, insect transmission and both intracellular and intercellular movement. The most important function of the CP is to form the shell in which genomic ssDNA is encapsidated. Replication of geminiviruses takes place in nucleus thus they require movement of virus from the cytoplasm into the nucleus along with cell- to- cell movement. The DNA B component encodes one gene, BV1 (the nuclear shuttle protein [NSP]) in the virion-sense and one gene, BC1 (the movement protein [MP]), in the complementary-sense. These gene products have been implicated in local and systemic spread, symptom development, host range determination and virus transmission by whiteflies.

The DNA-A and DNA-B components share little sequence similarity with the exception of a ~170nt stretch of sequence in the intergenic region (IR), termed the common region (CR) (Hanley- Bowdoin *et al.,* 1999). Although the CR sequence is usually almost identical in both components, there are examples where the CRs differ substantially between the cognate DNA-A and DNA-B components. Cotton leaf curl disease (CLCuD) was reported in the Sudan almost 80 years ago and has been a major constraint to cotton production in Pakistan for over a decade (Briddon and Markham, 2001b; Nour and Nour, 1964). The disease is associated with a search for additional components resulted in the isolation of a small circular ssDNA, referred to as DNA-1 (Mansoor *et al.,* 1999), that is representative of a new class of component associated with monopartite begomoviruses that are collectively referred to as alphasatellites (Briddon *et al.,* 2004). A database search showed a novel ssDNA component of approximately half the size of the helper begomovirus was isolated and shown to induce the yellow vein phenotype when re-introduced with AYVV into *Ageratum* (Saunders *et al.,* 2000). The component at that time was named DNA β (now known as Ageratum yellow vein betasatellite [AYVB]) because, in many respects, it functionally resembled the DNA-B component of bipartite begomoviruses. Transmission and propagation studies of both the components of the disease in *Ageratum* using the whitefly vector confirmed the etiology of the disease.

Comparisons of the nucleotide sequences of all betasatellite identified three absolutely conserved features: (1) a predicted stem loop structure with the loop sequence TAA/GTATTAC [the nonanucleotide sequence (NS)], contain a region of high sequence similarity referred to as the "satellite conserved region" (SCR). This is the regions where universal primers for betasatellites were designed that have proven to be a simple and robust method for the isolation of betasatellites (Briddon *et al.,* 2002), (2) an adenine- (A) rich region approximately 370 to 420nts upstream of the SCR. The function of A-rich region is not fully known. One of the hypotheses is that it might act as a "stuffer" to fulfill the size requirements of begomoviruses for movement and encapsidation. (3) A single positionally and sequence conserved gene encoded on the complementary-sense strand known as the *βc1* gene (Amin *et al.,* 2006; Briddon *et al.,* 2003a).

Methodology

1. Collection of plant samples:

The infected leaf samples were collected, from the different regions of North India on the basis of typical begomovirus symptoms i.e., yellowing of leaf veins, yellow mosaics patterns, curling of fractional or complete leaves, leaf distortion, chlorosis and decrease in leaf size and stunting in plants, kept in plastic bags labeled with permanent marker, carried on dry ice or liquid nitrogen and stored at -20°C until utilized.

2. Genomic DNA extraction:

Total DNA from leaf samples were extracted by using a CTAB (Cetyl Tri-methyl Ammonium Bromide) method (Saghai-Maroof *et al.*, 1984). The DNA pellet was washed with cold 70% ethanol twice, vacuum dried, and resuspended in 100 µl of TE buffer.

3. Estimation of DNA quantity and quality:

Concentration of DNA was estimated through NanoDrop (Cuvette free spectrophotometer) to check the quantity of DNA/µl for further study. DNA quality will also check by running the DNA samples on 0.6 % agarose gel using 1X TAE. The gel will stained with ethidium bromide (0.3 mg per ml) and read under UV light on a transilluminater or Gel-doc system.

4. PCR amplification of DNA:

For amplification of DNA by PCR a reaction mixture of 50µl containing 10pg- 1µg template DNA, 5µl 10X *Taq* polymerase buffer, 5µl 2mM dNTPs, 1.5mM MgCl2, 0.5µM of each primer and 1.25 units *Taq* DNA polymerase will prepared in a 0.25ml thin walled PCR tube. The reaction mixture was carry out in thermal cycler, using begomovirus specific universal primers against coat protein (CP) and β-satellite. This was confirming the molecular level infection of the begomovirus in plant samples.

5. Sequencing of DNA samples:

Sequencing of the amplified product was carried out by using ABI automated sequencer present at TCGA (The Centre for Genomic Application) New Delhi and Chromos Biotech Hyderabad.

6. Submission of sequences in NCBI and Homology search:

After sequencing the DNA sequence will submitted to NCBI-BankIt or Sequin. Homology search will carryout by using alignment tools (e.g., NCBI-BLASTn etc.).

7. Phylogenetic study:

Database search was carried out with BLAST*n* or with other BLAST tools (http://www.ncbi.nlm.nih.gov/BLAST). MEGA version 4.0 (http://www.megasoftware.net) was used for Pair wise and Multiple sequence alignments through its CLUSTAL- W program and Phylogenetic trees were constructed by using Neighbor-Joining algorithm.

8. Construction of clones:

A 750 nucleotides fragment corresponding to the betasatellite of begomovirus infected leaf was cloned in sense and anti-sense orientation with short intron. The resulted amplicon was cloned in the binary vector pCAMBIA 1300 (kindly provided by Ikegmai, Tohoku University, Japan) driven by strong *Cauliflower mosaic virus* (CaMV) 35S strong promoter and PolyA signal near right border of plasmid. To monitor silencing, down-regulation of GUS protein expression was perform by using the pBin121-derived expression construct.

9. Plant Transformation:

The resulting binary constructs were introduced into *A. tumefaciens* LBA4404 by electroporation with a Gene Pulser apparatus (Eppendoff, USA), and the later were used to develop transgenic *N. benthamiana*. For the transformation of *N. benthamiana*, plants were carried out as per leaf disk transformation method (Horsch *et al.,* 1985).

10. Agroinfiltration:

A grown culture of *A. tumefaciens* LBA4404 strain harboring the different pCAMBIA1300 and pBin121-derivative binary plasmids were used for agroinfiltration on abaxial side of non-transformed *N. benthamiana* leaf and transformed leave of *N. benthamiana* plants, incubate in growth chamber at 25°C for 48-76 hrs. Detection of GUS gene expression was done by histochemical GUS staining.

11. Screening of Transformed plants using PCR:

Total DNA was extract from young leaves of transgenic plants by CTAB method (Saghai-Maroof *et al.*, 1984). To confirm the transgene, PCR will carry out with specific primers.

12. Southern blot analysis of transformed plants:

Southern blotting was carried out as described (Sambrook *et al.*, 2001). Hybridization was performed using the intron from betasatellite gene (Wesley et al., 2001) present in the vector pCAMBIA-1300, labeled with DIG using an AlkPhos Direct Labelling and Detection System-kit (GE Healthcare) according to the manufacturer's instructions.

13. siRNA detection by Nothern-blot analysis:

Total RNA was extract from leaves of transgenic plants by the methods described by (Hamilton *et al.,* 2002). 50 µg of total RNA was separated on a 15% polyacrylamide (19:1) gel containing 7 M urea in 0.59 Tris–borate–EDTA (TBE). The gel was transferred to nylon membrane. To prepare the probes, pGEM-T/ βC1 plasmid was digested with *EcoRI* site located 340 bp downstream of the ATG codon of the βC1-gene. Transcripts were made from SP6 promoter. These transcripts were DIG-labeled according to the manufacturer's protocol (DIG Application Manual, Roche). Hybridization will perform overnight and signals were visualized by autoradiography on X-ray film at 80°C.

14. Publication of research results and thesis writing.

Publication of research results in National and International journals, books, symposiums and conferences.

Findings and concluding remarks

Over the last decade, the prevalence and severity of crops infecting begomoviruses have increased to epidemic proportions and consequently, begomoviruses became one of the major limitations for crops production throughout India. The sudden emergence of these whitefly-transmitted geminiviruses followed the introduction and rapid dispersal of *Bemisia tabaci.* As limited sources of resistance were available, this situation required the development of novel strategies for the disease control including the production of resistant cultivars which should be part of an integrated management approach. These management strategies should be based on a more detailed understanding of the pathosystems involved. Therefore, this PhD research focused on a comprehensive identification and characterization of the begomovirus species involved in the epidemics. Also a study of different aspects implicated in the complex virus-host interactions involving the RNA silencing response generated by begomovirus infection in plants was carried out.

The main conclusions from the results presented in this thesis are.

- A countrywide survey of cultivated crops, Ornamentals and weeds-infecting begomoviruses was conducted in **chapter 4.1** to identify the *begomoviruses* and their possible alternative hosts in India. The experimental host range of this virus includes vegetable and ornamental crops and some weed species, predominantly, but exclusively, in the family mainly *Solanaceae and Cucurbitaceae* which are widely distributed throughout the country.

From our results, we conclude that our findings concur with hypotheses formulated at the onset of the study. A wide range of weeds and other crops were observed to occur in both crop fields and surrounding ecosystem (Table 4.1.3). Our study also established that weeds and ornamental plants could act as alternative hosts to some begomoviruses that were identified during this study. These results present a good starting point for begomovirus diseases diagnosis in northern India; throw more light on the use of partial sequences to compare geminiviruses; and give sound begomovirus disease and vector management options.

- The molecular characterization of monopartite begomoviruses coat protein and betasatellites associated with different crop species, isolated throughout the country revealed the panorama of their distribution and genetic diversity, studies carried out during this thesis in **chapter 4.2.** Samples with typical begomovirus symptoms were collected in five different states, including the highly cultivated and crop growing areas of the country. Detailed analysis of these isolates established the existence of previously un-described begomovirus species.

Phylogenetic analysis placed these viruses in separate clades, distant from all the previously characterized begomoviruses. Interestingly, the manifestation of sequence analysis using CP genes and betasatellites of the monopartite begomovirus complexes suggested that inter-specific recombination has resulted in remarkable diversity among their geographical isolates.

- During these studies a species named Spinach yellow vein Sikar *virus* (SYVSV) was found to be widely dispersed in Northern regions of the country. The molecular properties of a representative isolate of this virus from Sikar of Rajasthan were further investigated in **chapter 5.1.** The full-length genome of SYVSV and associated β-satellite were cloned and sequenced. Phylogenetic studies based on the DNA-A sequences of begomoviruses placed SYVV- [Sik] together with other begomoviruses, SYVV- [Sik] shares 88% sequence identity with an isolate of *Papaya leaf curl virus (*PaLCuV) reported from the state of Pakistan, and referred to as SYVV- [Sik]. Extensive sequence comparisons suggest that isolates SYVV- [Sik] bear evidence of recombination events infecting Spinach acts as a natural host for *Papaya leaf curl virus*, undoubtedly pointing towards the begomovirus evolution. Although the disease caused by begomovirus species used in these pioneer works was not characterized previously, therefore to our knowledge this is the first report of molecular characterization and recombination study of Spinach-infecting begomovirus and their associated satellites molecules from India. Taken all together our observations suggest that the newly identified begomoviruses are native to Pakistan and were transmitted from weeds and wild plants into crops such as spinach by the introduced whitefly populations, effectively colonize this crop.

- **In chapter 5.2** for monitoring of monopartite begomovirus in some localities in the Rajasthan Republic, 20 *Cyamopsis tetragonoloba* (guar bean) samples showing curling symptoms in their leaves, collected separately from different localities of cereal production (guar bean) during 2012. CTAB-DNA extraction methods were used for the detection of begomovirus in the infected leaves of guar bean, were on the bases of PCR analysis to be reliable. The presence of begomovirus was confirmed in all samples analyzed.

To characterize begomovirus from *C. tetragonoloba,* full-length DNA-A & betasatellites genomic molecule of begomovirus had been cloned and sequenced. The complete DNA-A of Radish leaf curl virus (RaLCuV) and Radish leaf curl betasatellites (RaLCuB) were determined to be 2,747 and 1,367 nucleotides respectively. The phylogenic analysis indicates that both, DNA-A & betasatellites shared the highest sequence identity (99%) with *Radish leaf curl virus* reported from northern art of India. To our knowledge, this is the First report of a betasatellite

associated with Radish leaf curl virus infecting new host *Cyamopsis tetragonoloba* (Guar bean) Rajasthan Republic in India

- Additional to introgression of natural sources of resistance, alternative biotechnological approaches for resistance in transgenic plants have been investigated to improve begomovirus resistance. The most commonly employed strategies deal with silencing of the βC1 gene which seems to be the gene of choice because of its pivotal requirement for viral replication, studies carried out in **chapter 6** during this thesis.

RNA silencing is a regulatory mechanism that involves the degradation of RNA in a sequence specific manner and in plants is involved in antiviral response. In chapter 6 the RNA silencing response generated in plants infected by CroYVMV was examined. Upon infection with CroYVMV, the RNA silencing machinery in host plant such as *N. benthamiana* was triggered and a population of siRNAs specific to CroYVMV was generated. Concomitantly, a decrease in the viral DNA levels was observed, thus suggesting that indeed DNA viruses are sensitive to RNA silencing, possible by targeting the viral mRNAs. Certain plant viruses encode suppressors of posttranscriptional gene silencing (PTGS), an adaptive antiviral defense responses that limits virus infection. A novel single stranded DNA of approximately ~1350 nucleotides in length, termed DNA-β, has been identified to associate with *Croton yellow vein mosaic begomovirus* (CYVMV) in croton plants. The expression of genes from DNA-β requires the begomovirus for replication, encapsidation, insect transmission and movement in plants.

Most established RNA silencing suppressors proteins have previously been implicated in virulence and reported as symptom determinants. Therefore, the silencing suppression potential of βC1 genes was investigated. To this end, the two most commonly used tests, i.e. the local *Agrobacterium* transient assay and the transgene silencing reversal assay were employed. In *N. benthamiana,* the primary targets corresponded to the βC1 gene of betasatellites. Aiming to generate virus resistant plants, the natural RNA silencing reaction to begomovirus (CYVMV) infection in *N. benthamiana* was exploited. For this purpose, transgenic plants with an intron-hairpin construct expressing dsRNA corresponding to the most targeted portions of the viral genome were produced. Finally in this chapter 6, the possible role of RNA silencing in begomovirus infection and the perspectives of their control by using biotechnological strategies are discussed.

Signifiance of Works :

(a) Constructive climatic condition of Northern India supports the high population of *Bemisia tabaci* (whitefly). This study gives the information about the natural host plants and vector capacity for transmission of begomoviruses in Northern states.

(b) occurrence of begomoviruses in wild species and the role of those plants help in understanding the epidemiology and evolution of begomoviruses

(c) Information about role of individual Begomovirus genes in pathogenic determinant and resistant development.

(d) Viruses or part of the viruses may be used to develop plant expression vector to produce high value product (such as medical vaccines) known as biopharming.

(e) Resistance is the ultimate aims of this type study to reduce the losses caused by these pathogens, clones of begomoviruses are likely to be major output of this study which will provide greatest benefits to agriculture.

(f) In the present economical scenario, with increasing demand of staple food (such as wheat and rice), potential benefits of such technologies for agriculturally based country like India to improve economy of the nation.

Future Prospects :

(a) From the survey conducted on Nothern region of India, it is quite evident that the complexity of begomoviruses and DNA-satellites in this region is high, and our knowledge needs to be broadened further with more extensive surveys including a wider range of geographical areas.

(b) Single and mixed infections by begomoviruses and their recombinants are probably responsible for new epidemics in horticultural crops. Little information is available about identity and relationships between the viruses involved in these epidemics in most crops and more studies would allow an increase of our knowledge on the variability and evolution of the begomoviruses in the New World.

(c) More studies are necessary to investigate the occurrence of begomoviruses and satellites on alternative hosts, other than crops.

(d) Resistant crop varieties seem to be necessary for slowing down the epidemics, but production of such varieties will be difficult due to the high diversity of begomoviruses infecting these crops.

(e) There is a need to investigate the effects of mutations of virus-encoded genes on begomovirus-associated satellites.

(f) There is a vital need to introduce or breed crops with broad-spectrum resistance to cope with possible emergence of new begomovirus complexes all over the world mainly in Asia.

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